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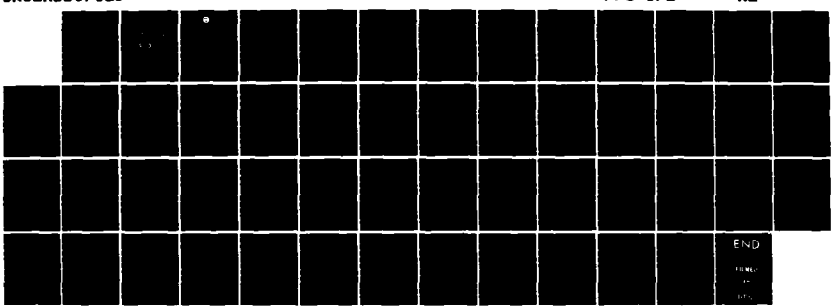

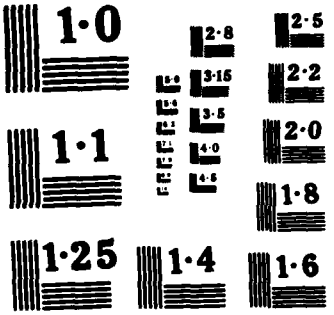

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Technical Report 930

CHEMICAL CHARACTERIZATION OF COMPOUNDS RELEASED BY MARINE MAMMALS

MG Ceruti
Code 512

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NOSC

NAVAL OCEAN SYSTEMS CENTER
San Diego, California 92152

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ADMINISTRATIVE INFORMATION

The work reported here was performed between January 1981 and March 1983 as a part of the Marine Animal Capabilities Program, sponsored by the Naval Sea Systems Command under Program Element 62759N.

This report summarizes documentation of some chemical analyses submitted by Dr PV Fennessey, Associate Professor of Pediatrics and Pharmacology and Susan S. Tjoa at the University of Colorado School of Medicine's Mass Spectrometry Research Resource, Denver, Colorado under contract numbers N66001-81-M-A236 and H66001-82-M-3175. Dr Fennessey and Ms Tjoa provided several enlightening discussions on results and analytical techniques.

The author extends gratitude to Dr PE Nachtigall and WA Friedl of Code 512 for their initiation, interest and support of this work; RW Hall, KV Keller and JL Richards of Code 512, and GA Peiterson for their assistance in sample collection; RH Brady of Code 446 for his editorial contributions; and K Wright, Code 4473, for her excellent work in literature searches.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Excretions, secretions and glandular extracts from marine mammals were analyzed chemically by gas chromatography and mass spectrometry to identify chemical constituents which may be involved in marine mammal chemoreception.

The results of acidic, neutral and basic fractions of urine, feces, prostate gland extract and semen from male dolphins, Tursiops truncatus; of a urine sample from a female dolphin, Tursiops truncatus; and of one fecal sample from a male California sea lion, Zalophus californianus, are presented.

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The acidic and neutral fractions of a perianal gland secretion and fecal sample from the same male dolphin; and a fecal sample from a female Tursiops truncatus also are presented.

Various acids, esters, sugars, alcohols, steroids and nitrogen-containing compounds were in the samples. Taste and/or toxicity data were available for 25 compounds detected in the samples. Some sugars, some alcohols and glycine are not expected to be toxic.

Several compounds are noted as suitable stimuli for chemoreception experiments. These are lactic, phosphoric and succinic acids; glycine; urea; mannose; glycerol; inositol; arabitol; erythritol; mannitol; sorbitol; xylitol; erythrose; galactose; glucose; lactose; xylose; indole and skatole.

CONTENTS

TABLES

OBJECTIVE

Identify several chemical compounds found in the urine, perianal gland secretion, prostate gland, semen and/or feces of marine mammals which could be detected chemoreceptively. Provide information on the taste of appropriate chemicals and recommend safe levels of exposure for marine mammals in chemoreception experiments.

RESULTS

1. The samples contained various acids, bases, steroids, polyhydric alcohols, phosphates, sugars, esters and neutral compounds. Not all constituents occurred in each sample.

2. All samples had constituents which could be used as stimuli in marine mammal taste reception studies.

3. Urine samples from a female Pacific bottlenose dolphin and a male Atlantic bottlenose dolphin had different constituent compounds.

4. Fecal samples from two male and one female Atlantic bottlenose dolphins also differed in chemical composition.

5. Some constituent compounds are not considered toxic. Safe concentrations of constituents which could be used in taste reception experiments can be estimated from laboratory studies on humans or animals or from concentrations used in foods or drugs prepared for human consumption.

6. Major components of marine mammal samples were similar to components from human controls.

7. High-level contamination from some plastic collection devices may have masked a large number of compounds present at low levels for some samples.

RECOMMENDATIONS

1. For marine mammal chemoreception experiments, start with the following solution concentrations: 0.7% lactic acid, 0.05% phosphoric acid, 0.05 M urea, 0.02 M inositol, 2.0 M glycine, 2.8 M sorbitol, 1.0 M xylitol, 2.5 M galactose, 2.0 M glucose, 0.6 M lactose, 2.5 M xylose, 0.01% indole and 0.005% skatole.

2. Test glycerol, erythritol, arabitol, mannitol, mannose and erythrose for taste reception using solutions of 10%.

3. Avoid p-hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic, 3,5-dihydroxybenzoic, palmitic, stearic, oleic, myristic and arachidic acids and cholesterol because they are inappropriate stimuli for chemoreception experiments due to toxicity or insolubility in water.

4. Use specially cleaned glass collection apparatus and techniques to allow analytic identification of low-concentration constituents.

INTRODUCTION

Many terrestrial mammals use chemoreception, particularly olfaction, during feeding, territorial demarcation and reproduction (ref 1, 2). Marine mammals also may have some chemoreceptive capabilities (ref 3-5). Except for Soviet literature, chemoreception studies are mainly histological and anatomical (ref 6). Behavioral observations indicate that cetacea may be sensitive to chemical signals and the presence of blood (ref 3, 4).

Soviet studies tested a variety of chemicals that had a strong taste or smell, and porpoises and dolphins indicated detection either physiologically or behaviorally (ref 2, 3, 7-10). Evidence indicates that dolphins' chemoreception sensitivity to sugar, salt and some acids may be less than that of terrestrial mammals (ref 8).

Some compounds in marine mammal excretions and secretions may be chemoreceptively active (ref 3, 4, 7, 8). Table 1 summarizes the results of Kuznetsov's chemoreception experiments (ref 7-10). The harbor porpoise (Phocoena phocoena) can detect protein metabolites such as trimethylamine and skatole in seawater solutions at concentrations on the order of 10^{-6} molar (ref 7, 10).

Soviet literature suggests that the perianal glands of male toothed whales may be involved in transmitting chemical signals directly into the water (ref 8).

In 1980, Naval Ocean Systems Center (NOSC) scientists began testing California sea lions' (Zalophus californianus) and bottlenose dolphins' (Tursiops truncatus) taste reception abilities. This study supports those tests by identifying possible chemoreceptively active compounds in marine mammal excretion and secretion samples.

SUBSTANCE	SOLVENT	CONCENTRATION	REF
Trimethylamine	Seawater	0.2%, 8.5×10^{-6}M	7,10
Trimethylamine	Seawater	0.2%	6
β-Phenylethyl Alcohol	Seawater	0.04% *	8
Indole	Seawater	0.01%	7,8
Indole	Fresh Water	10^{-6}M	10
Skatole	Seawater	1.7×10^{-6}M	10
Camphor	Seawater	0.01%	7,8
Camphor	Seawater	3×10^{-6}M	10
Quinine Chloride	Seawater	1×10^{-4}M	10
Quinine Chloride	Fresh Water	1×10^{-5}M	10
Hydrochloric Acid	Seawater	0.15 M	10
Hydrochloric Acid	Fresh Water	0.1 M	10
Caproic Acid	Fresh Water	5×10^{-5}M	10
Citric Acid	Seawater	0.2 M	10
Citric Acid	Fresh Water	0.05 M *	10
Oxalic Acid	Seawater	0.7 - 2.5% *	8
Oxalic Acid	Fresh Water	0.03 M	10
Picric Acid	Fresh Water	2×10^{-5}M *	10
Valeric Acid	Seawater	0.1 - 0.5% *	8
Valeric Acid	Fresh Water	1×10^{-5}M *	10
Valeric Acid	Seawater	1×10^{-4}M	10
Male Tursiops Urine	Seawater	Dilution of 10^{-2}	10

Table 1. Summary of substances detected by cetacea. Solvent indicates the carrier in which substances were dissolved. Concentrations are either a single value or a range over which chemoreceptive detection occurred. Threshold values are indicated by an asterisk.

CHEMICAL ANALYSIS

MATERIALS AND METHODS

One sample of urine and four of semen (sperm and/or seminal fluid) were obtained from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-583). One fecal sample from a male California sea lion Zalophus californianus (Zc-532) was collected in a plastic container. The dolphin samples were transferred immediately to separate glass vials for storage and shipping. One urine sample from a female dolphin, Tursiops truncatus (Tt-605), was collected in a plastic cup.

The following samples were collected in pre-cleaned sterilized glass jars or test tubes. During necropsy, the prostate gland was dissected from a male dolphin (Tt-026) and placed in a glass jar. Fecal and blood samples also were collected from the same animal during the dissection. A milky white perianal gland secretion was collected from a live male dolphin (Tt-042). Fecal samples also were obtained from two dolphins, Tt-594, a female, and Tt-042, a male.

All samples were frozen immediately. Between several days and three months later, they were sent to the University of Colorado Health Sciences Center for analysis by Dr PV Fennessey and Susan S. Tjoa. The samples were packed in dry ice during shipment.

ANALYSIS

All except three samples were separated into three organic fractions (acidic, neutral or basic) by solvent extraction and analyzed by gas chromatography and mass spectrometry (ref 11, 12). Details of the technique are described in a report obtained under contract for the analyses (ref 13). Copies are available from the author upon request.

For the samples from Tt-583 and Zc-532, one to five ml of rinse water from an empty plastic bag, a plastic container, and an empty glass vial similar to those used in sample collection and shipment were analyzed in the gas chromatograph for background contamination. Malonic acid was the internal standard and $C_{24}H_{50}$, a hydrocarbon, was the external standard.

Substances were identified from retention time in the chromatograph and from charge-to-mass ratios in the spectrometer. Relative amounts of components were indicated by the area under the chromatographic curves (ref 11).

Four types of gas chromatographic columns were used to separate the components. Packed column types, OV-22 and OV-1, were used to analyze the samples from Tt-583 and Zc-522. Capillary gas columns, types SE-30 and OV-101, were used to analyze tne acidic and neutral fractions of the other samples. The basic fractions of the urine from Tt-605 and of all samples from Tt-026 also were analyzed with the SE-30 column.

RESULTS

The results from analyses of dolphin urine, semen, prostate glandular extract, blood, perianal gland secretion, feces and of sea lion feces are in tables 2-11. The amounts of perianal gland secretion from Tt-042 and feces from Tt-594 and Tt-042 were too small to permit analyses of the base fractions. Therefore, data for only the acidic and neutral fractions are shown in tables 7, 8 and 9 for those samples. All tables also indicate the estimated relative amounts of the various constituents. The chromatographic column type used to obtain the data also is indicated in the tables.

Some compounds, such as long chain fatty acids and/or lipids, sugars and polyhydric alcohols, were found in many samples: for example, palmitic acid (7 samples), stearic acid (10 samples) and oleic acids (5 samples).

Phosphoric acid was found in at least five samples. It was the most abundant component in the acidic fractions of (a) two semen samples from Tt-583, (b) the fecal sample from Tt-594 and (c) the fecal sample of Zc-532. Phosphoric acid also may have been in the neutral fraction of the perianal gland sample (table 7).

Polyhydric alcohols also were abundant in many samples. Except for one semen sample, inositol was found in every sample. Inositol was the most abundant component in the various fractions of other semen samples, prostate gland extracts and blood. Mannitol, another polyhydric alcohol, occurred in six samples. Mannitol was most abundant in neutral fractions of semen sample number one (table 4), the prostate gland extract (table 5) and dolphin feces (table 8).

Several sugars were detected. Glucose occurred in at least four samples. Erythrose was the most abundant neutral component in the fecal sample from Tt-026.

Some compounds were identified only once in these samples. Urine from Tt-583 had six acids and at least one neutral compound not found in any other sample. Similarly, the fecal sample from Zc-532 contained one acid, one sugar and three bases unique to the samples analyzed. Talose, the most abundant neutral component, is an example (table 11).

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		SE-30	OV-101		SE-30
Palmitic acid	0.042	1.0	Palmitic acid or a palmitate ester	0.33	0.91	Palmitic acid or a palmitate ester	1.0
Stearic acid	0.042	0.0	Stearic acid or a stearate ester	0.45	1.0	Stearic acid or a stearate ester	0.23
p-hydroxy-3-phenyl-lactic acid	0.0	1.0	Erythritol	0.0	0.080		
p-hydroxyphenyl-acetic acid	1.0	0.0	Inositol	1.0	0.98		
Threonic acid	0.94	0.0	Cholesterol	0.0	0.074		
A phosphate	0.0	0.35	3-β-hydroxy-5-stigmastene	0.0	0.086		

Table 2. Principal chemical components in the acidic neutral and basic fractions of a urine sample from a female Pacific bottlenose dolphin, Tursiops truncatus, (Tt-605). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	OV-22		OV-1		OV-22
Succinic acid	0.4	Arabitol	0.13	Urea	1.01
Phosphoric acid	0.5	Ribitol	0.11		
Threonic acid	1.0	Inositol	0.02		
Erythronic acid	0.8	Erythrose	1.0		
Threitol	0.2	Mannose	0.09		
Erythritol	0.3	Galactose	0.06		
Lactic acid	0.4	Glucose	0.09		
p-Hydroxy-3-phenyllactic acid	0.3	Lactose	0.04		
β-Hydroxybutyric acid	0.5	Glucosamine (?)			
β-Hydroxyvaleric acid	0.5	Other sugars (?)			
2-Keto-3-methyl-butyric acid	0.4				

Table 3. Principal chemical components in the acidic, neutral and basic fractions of a urine sample from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-583). The estimated relative abundances are indicated with those of the most abundant component equal to one. The chromatographic column type is indicated for each fraction. Question marks indicate tentative assignments.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE			NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE			BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		
	OV-22				OV-1				OV-22		
	sample #				sample #				sample #		
	1	2	3		1	2	3		2	3	4
Stearic acid	0.01	0.03	0.5	Inositol	0.0	1.0	1.0	Urea	0.33	1.0	1.0
Oleic acid	0.0	0.03	0.0	Galactose	0.0	0.0	0.33	A palmitic acid or a palmitate ester	0.55	0.0	0.0
Citric acid	0.12	0.0	0.0	Glucose	0.0	0.0	0.44				
Glycine	0.03	0.0	0.0	Mannitol	1.0	0.0	0.0				
Inositol	0.06	0.0	1.0					Stearic and oleic acids or stearate and oleate esters	1.0	0.0	0.0
Glycerol	0.22	0.0	0.0								
Phosphoric acid	1.0	1.0	0.5								
α-Glycerophosphate	0.7	0.0	0.5								
β-Glycerophosphate		0.0	0.33								
Cholesterol (?)											

Table 4. Principal chemical components in the acidic, neutral and basic fractions of four semen samples from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-583). The estimated relative abundances are indicated with those of the most abundant component of each sample equal to one. The chromatographic column type is indicated for each fraction. Question mark indicates tentative assignment.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		SE-30	OV-101		SE-30
Palmitic acid	0.081	0.0	Sorbitol	0.002	0.45	Palmitic acid or a palmitate ester	1.0
Stearic acid	0.11	0.0	Mannitol	0.29	0.48		
Oleic acid	0.031	0.0	Ribitol	0.0	0.086	Stearic acid or a stearate ester	0.97
Citric acid	0.045	0.0	Arabitol	0.0	0.031		
α-Glycerophosphate	0.15	0.0	Inositol	1.0	1.0	Oleic acid or an oleate acid	0.47
β-Glycerophosphate	0.021	0.0	Palmitic acid and a sugar (mixture) or palmitate ester and a sugar (mixture)			p-hydroxyphenyl-lacetic acid or a p-hydroxy-phenylacetate ester	0.090
Inositol	0.040	1.0					
Mannose or glucose phosphate	0.042	0.0					
A sugar	1.0	0.0		0.038	0.0	Phosphate	0.041
A sugar phosphate	0.85	0.0					

Table 5. Principal chemical components in the acidic, neutral and basic fractions of an extract from the prostate gland of a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction for each chromatographic column type, equal to one.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		SE-30	OV-101		SE-30
Palmitic acid	0.14	0.0	Palmitic acid or a palmitate ester	1.0	0.0	Palmitic acid or a palmitate ester	1.0
Stearic acid	1.0	0.0	Stearic acid or a stearate ester	0.10	0.0	Stearic acid or a stearate ester	0.63
Phosphoric acid	0.041	0.0					
Inositol	0.68	0.0	Mannitol	0.0	0.19	Oleic acid or an oleate ester	0.22
			Sorbitol	0.0	0.24	Myristic acid or a myristate ester	0.29
			Inositol	0.0	1.0	Phosphate	0.18
			Arabitol	0.0	0.046	Cholesterol	0.056

Table 6. Principal chemical components in the acidic neutral and basic fractions of a blood sample from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one. In the acidic fraction, no compounds were detected with the OV-101 column.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	
	SE-30		SE-30	OV-101
Palmitic acid	0.89	Palmitic acid or a palmitate ester	0.0	0.86
Stearic acid	1.0	Stearic acid or a stearate ester	0.0	1.0
Oleic acid	0.13	Phosphoric acid or a phosphate	1.0	0.0
3,4-Dihydroxybenzoic acid	0.06			
3,5-Dihydroxybenzoic acid	0.04			
Isocitric acid	0.23			
Inositol	0.28			

Table 7. Principal chemical components in the acidic and neutral fractions of a perianal gland secretion sample obtained from a male Atlantic bottlenose dolphin Tursiops truncatus (Tt-042). The estimated relative abundances are indicated with those of the most abundant component in each fraction for each chromatographic column type equal to one.

ACIDIC FRACTION	ESTIMATED ABUNDANCE		NEUTRAL FRACTION	ESTIMATED ABUNDANCE	
	SE-30	OV-101		SE-30	OV-101
Palmitic acid	0.74	0.0	Stearic acid or a stearate ester	0.0	0.06
Stearic acid	1.0	0.0	Glucose	0.0	0.73
Oleic acid	0.11	0.0	Erythrose	0.30	0.99
Isocitric acid	0.22	0.0	Mannitol	0.38(?)	1.0
p-Hydroxyphenylacetic acid	0.13	0.0	Inositol	0.39	0.0
Phosphoric acid	0.0	1.0	A sugar	0.0	0.11
			A sugar-phosphate	0.095	0.0
			Another sugar-phospate	1.0	0.0

Table 8. Principal chemical components in the acidic and neutral fractions of a fecal sample from a female Atlantic bottlenose dolphin, Tursiops truncatus (Tt-594). The estimated relative abundances are indicated with those of the most abundant component of each column type, equal to one. Question mark indicates tentative assignment on the SE-30 column.

ACIDIC FRACTION	ESTIMATED ABUNDANCE		NEUTRAL FRACTION	ESTIMATED ABUNDANCE	
	SE-30	OV-101		SE-30	OV-101
Palmitic acid	1.0	0.0	Palmitic acid or a palmitate ester	0.81	0.0
Stearic acid	0.55	0.0	Stearic acid or a stearate ester	1.0	1.0
Oleic acid	0.18	0.0	Gluconolactone	0.0	0.49
Myristic acid	0.0	1.0	Erythrose	0.082	0.0
			Erythritol	0.0	0.49
			Xylitol (?)	0.41	0.0
			Mannitol	0.61	0.0
			Inositol	0.13	0.16

Table 9. Principal chemical components in the acidic and neutral fractions of a fecal sample obtained from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-042). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one. Question mark indicates tentative assignment.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		SE-30	OV-101		SE-30
Palmitic acid	0.67	1.0	Palmitic acid or a palmitate ester	0.27	0.24	Palmitic acid or a palmitate ester	1.0
Stearic acid	1.0	0.88	Mannitol	0.42	0.02	Stearic acid or a	
Myristic acid	0.0	0.25	Sorbitol	0.0	0.02	Oleic acid or an leate ester	0.81
α-Glycerophosphate	0.15	0.0	Inositol	1.0	1.0	Myristic acid or a myristate ester	0.11
Inositol	0.032	0.0	Xylitol, ribitol or xylose (?)	0.0	0.38	Sebacic acid or a sebacate ester	0.51
A phosphate	0.0	0.38	Erythrose	0.67	0.43	Glycol-1-palmitate	0.065
			A phosphate	0.014	0.0		

Table 10. Principal chemical components in the acidic, neutral and basic fractions of a fecal sample from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column equal to one. Question mark indicates tentative assignment.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	OV-22		OV-1		OV-22
Phosphoric acid	1.0	Glucose	0.75	Glycerol	0.06
3-(4-Hydroxyphenyl) propionic acid	0.3	Galactose	0.05	Palmitic acid or a palmitate ester	1.0
		Talose	1.0		
		Xylitol	0.1	Arachidic acid or an arachidate ester	0.29
				Stearic and oleic acid or stearate and oleate esters	0.16
				Tyramine	0.59
				Tryptamine	0.27

Table 11. Principal chemical components in the acidic, neutral and basic fractions of a fecal sample from a male California sea lion, Zalophus californianus (Zc-532). The estimated relative abundances are indicated those of the most abundant component equal to one. The chromatographic column type also is indicated for each fraction.

At least seven other chemicals from five other samples were detected only once in this series.

Urea, the only component found in the basic fraction of the urine sample from the male dolphin (table 3), also occurred in the basic fraction of three semen samples.

DISCUSSION

The majority of compounds detected in the acidic and basic fractions of the urine, semen and prostate gland samples also occur in samples from other mammals, including humans (ref 14-68). Citric acid and glycine are common in mammalian semen. Human semen also contains inositol (ref 43, 44, 49-52).

Glycerol was in the basic fraction of sea lion feces, and several long chain fatty acids occurred in the basic fractions from dolphin urine, semen, prostate gland extract, feces and blood. These acids may have been attached as esters to glycerophosphate amine compounds in the samples (ref 13).

Fatty acids also occurred in the neutral fractions of dolphin urine, prostate gland extract, feces and blood. These acids may have been present in their lipid forms (as esters) in the samples and thus would tend to a more neutral than acidic character in the analyses. A similar transformation could be true for the acid/esters mentioned above. Because such ester transformation is possible, the exact original compounds present in the neutral and basic fractions of some samples were indeterminable.

Several compounds in the acidic and neutral fractions of the blood sample (table 6) may be involved in cetaceans' purported chemoreceptive sensitivity to blood (ref 3, 4). These compounds are discussed below in the section entitled "Taste and Toxicity."

The chemical analysis of the perianal gland secretion (table 7) is the first for marine mammals (ref 69). The perianal gland secretion from the dolphin was milky white and possibly contained lipids (esters of fatty carboxylic acids) and carboxylic acids. Lipids have been reported in perianal gland secretion from the black-tailed prairie dog, Cynomys ludovicianus (ref 70), and carboxylic acids occur in anal scent pockets and glands of the Indian mongoose, Herpestes auropunctatus, and of the red fox, Vulpes vulpes, respectively (ref 1, 71). Moreover, green and white secretions from otter (Lutra lutra) anal scent sacs also contain lipids and fatty acids (ref 2).

LIMITATIONS OF ANALYTICAL TECHNIQUES

Background contamination from the plastic bags and cup used in sample collection interfered mainly with neutral fraction analyses. Thus, the neutral fractions reported for samples Tt-583 and Zc-532 indicate only major components. Urine was collected from Tt-605 in a plastic cup, but analytic interference was less than from the plastic bags. All other samples were collected in specially cleaned glassware which was obtained from the analytical laboratory in Colorado. Samples collected in the glassware were free from container contamination. Sample collection techniques are discussed in appendix A.

Some extremely volatile compounds may have escaped from the samples shortly after collection. Moreover, the analyses may not have detected some compounds in the samples because gas chromatography measures only volatile derivatives. Although only about 20 percent of all substances present in materials of biological origin are volatile enough to be detected with gas chromatography, the compounds which are detected are likely to be involved in mammalian chemoreception (ref 27).

The analytic techniques are limited further if two constituents have the same retention time in the chromatograph, or the same charge-to-mass ratio in the spectrometer. Such compounds are detected as a single substance so the presence of one is masked. However, the probability that two constituents would have the same retention time and charge-to-mass ratio is low.

The relative amounts of compounds separated on the same chromatographic column type can be compared fractionally and across samples. However, fractions separated on different column types cannot be compared because of different chemical response characteristics of the columns. Further, different column types were used at different times during the analysis and sample composition could have changed but was not tested.

TASTE AND TOXICITY

BACKGROUND

The qualitative taste information in this section is from experiments and human observations. Some substances, such as indole and skatole, were toxic to terrestrial mammals in laboratory tests (ref 72-91). However, the toxicity is dose-dependent and may be species specific. Kuznetsov used indole and skatole in dolphin chemoreception experiments (ref 6-8).

RESULTS

Tables 12 through 15 summarize taste and toxicity information on 27 of the principal compounds identified in the marine mammal products tested (ref 92-132). An indole derivative was identified in the basic fractions, so the toxicity of some nitrogenous heterocycles is presented. Taste and toxicity information is presented for acidic, neutral and basic compounds. Compounds for which no relevant information was available are not included in the tables.

TASTE AND TOXICITY OF SOUR AND/OR BITTER SUBSTANCES

The five compounds listed in table 12 are discussed below.

Lactic Acid (ref 92-95, 97)

Lactic acid may be well-suited for chemoreception studies. It is common naturally and occurs in foods such as sauerkraut, pickles, cheese, beer and sour milk. It is considered Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA).

Lactic acid in concentrations from 0.1 to 0.7 percent is a food additive and preservative. Food-grade lactic acid has a mild, fruity flavor resembling yeast, lemon and sour apple. Lactic acid (0.0064 M) was used in taste reception experiments on human subjects.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
Lactic Acid	$CH_3CHOHCO_2H$	90.1	Very soluble (92)	Acrid, fruity (93) Salty (94)	Moderate (95)	General purpose food additive GRAS (93,95)
Succinic Acid	$COOH(CH_2)_2CO_2H$	118.1	Moderate (96)	Tart, bitter (96, 97)	Moderate (95,96 98)	General purpose food additive (93,95)
Phosphoric Acid	H_3PO_4	98.0	Very soluble (99)	Sour (100)	Moderate (95)	General purpose food additive (95,100)
Urea	$CO(NH_2)_2$	60.1	Very soluble (99)	Bitter, sour (94,101, 102)	Toxic in concentrated solutions (107)	Veterinary nutritional factor diuretic and antiseptic (96,97)
β-D-Mannose*	$C_6H_{12}O_6$	180.2	Very soluble (99)	Bitter (99,103,104)	N/A	The α-anomer is sweet (103)

Table 12. Summary of physical properties and toxicity information for sour and/or bitter compounds identified in the urine and semen samples from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-583). Parenthetical numbers are literary references. *While the presence of particular isomer(s) of mannose could not be determined in the analysis, different anomeres of mannose have different tastes.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
Glycine	$H_2NCH_2CO_2H$	75.1	Very soluble (95,99)	Sweet (108,109, 110)	N/A	Principal amino acid in sugar cane (95) 0.8 times as sweet as sucrose (111)
Glycerol	$HOCH_2CH(OH)-CH_2OH$	92.1	Very soluble (109)	Sweet (109)	Low (112)	Food sweetener, about 0.6 times as sweet as cane sugar (96,109)
Inositol	$C_6H_6(OH)_6$	180.2	Very soluble (99)	Sweet (96,105)	N/A	B vitamin (113,114)
Arabitol	$C_5H_7(OH)_5$	152.2	Very soluble (96)	Sweet (96)	N/A	N/A
Erythritol	$C_4H_6(OH)_4$	122.1	Very soluble (96)	Sweet (96)	N/A	About twice as sweet as sucrose (96)
Mannitol	$C_6H_8(OH)_6$	182.2	Very soluble (96)	Sweet (96)	N/A	Excipient and diuretic (96)
Sorbitol	$C_6H_8(OH)_6$	182.2	Very soluble (96)	Sweet (96,115)	Low (96,116)	Food additive, about 0.6 times as sweet as sucrose (95,96)
Xylitol	$C_58_7(OH)_5$	152.2	Very soluble (99)	Sweet (105,115)	N/A	Has slight unpleasant taste component (115)

Table 13. Summary of physical properties and toxicity information for one sweet amino acid and sweet polyhydric alcohols identified in excretions and secretions of marine mammals. Parenthetical numbers are literary references.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
L-Erythrose	$C_4H_8O_4$	120.1	Soluble (96,99)	Sweet (96)	N/A	Syrup (96)
α-D-Galactose	$C_6H_{12}O_6$	180.2	Very soluble in hot water (96,99)	Sweet (105,117, 118,119)	N/A	Medical use: liver function test (96,120)
D-Glucose	$C_6H_{12}O_6$	180.2	Very soluble (99)	Sweet (117,118, 119,121)	N/A	L-glucose is salty (121); bitter side taste (120); 0.74 times as sweet as sucrose (96)
β-Lactose	$C_{12}H_{22}O_{11}$	342.3	Very soluble (99)	Faintly sweet (96,119,121, 122)	N/A	Milk sugar; β-form sweeter than α-form (96)
α-D-Mannose	$C_6H_{12}O_6$	180.2	Very soluble (96,99)	Sweet (103)	N/A	The β-anomer is bitter (103)
D-Xylose	$C_5H_{10}O_5$	150.1	Very soluble (96,99)	Very sweet (96,105,118 123)	N/A	Wood sugar (95,98) diabetic food (96, 99)

Table 14. Summary of physical properties and toxicity information for sweet sugars identified in acidic and neutral fractions of excretions and secretions of marine mammals. Parenthetical numbers are literary references. The isomers with the sweetest tastes are listed here, although particular isomers were not specifically identified in the analysis.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
p-Hydroxy-3-phenyllactic acid	$C_9H_{10}O_4$	172.2	N/A	N/A	N/A	Experimental carcinogen (95,124)
3,4-Dihydroxybenzoic acid	$C_6H_3(OH)_2CO_2H$	154.1	Slightly soluble (98)	Probably bitter or sour (125)	N/A	Mutagen (116,126,127) Flavor threshold = 30 ppm (106)
3,5-Dihydroxybenzoic acid	$C_6H_3(OH)_2CO_2H$	154.1	Soluble in hot water (99)	Bitter, sour (125,106)	N/A	Flavor threshold = 90 ppm (106) Potential carcinogen (126)
Palmitic Acid	$CH_3(CH_2)_{14}CO_2H$	256.4	Insoluble (98)	N/A	N/A	Tumorigen and mild skin irritant (116)
Stearic Acid	$CH_3(CH_2)_{16}CO_2H$	284.5	Insoluble (99)	N/A	Oral-low IV-high (95,128)	Food additive to 4000 ppm (128)
Oleic Acid	$CH_3(CH_2)_7CH{:}CH-(CH_2)_7CO_2H$	282.5	Insoluble (99)	Smooth, unpleasant (111)	N/A	
Myristic Acid	$CH_3(CH_2)_{12}CO_2H$	228.4	Insoluble (99)	N/A	Orally non-toxic (129)	Found in nutmeg, palm seeds and milk fats (96)
Arachidic acid	$CH_3(CH_2)_{18}CO_2H$	312.5	Insoluble (99)	N/A	N/A	Found in peanut, vegetable and fish oils (96)
Cholesterol	$C_{27}H_{46}O$	386.7	Insoluble (99)	N/A	N/A	

Table 15. Summary of physical properties and toxicity information for compounds insoluble in water and/or considered unsafe. Parenthetical numbers are literary references.

Succinic Acid (ref 95-98, 115, 124, 130-132)

Succinic acid has a tart taste, but no odor. Aqueous solutions are slightly bitter and the taste buildup is slow. Succinic acid occurs naturally in broccoli, rhubarb, beets, asparagus, fresh meat extracts and cheese. It is also a general-purpose food additive.

Low concentrations of succinic acid have no systemic toxic effects. It is a common metabolite excreted in human urine.

Phosphoric Acid (ref 96, 97, 100, 133, 134)

Phosphoric acid, which has a sour taste, occurs naturally in fruits and fruit juices. Phosphoric acid is GRAS; soft drinks contain about 0.01 to 0.05 percent phosphoric acid. Some food processors use phosphoric acid to clarify sugar-bearing juices. In chemoreception experiments, 0.01 to 0.05 percent solutions of phosphoric acid can be used. Inhalation of phosphoric acid vapor should be avoided.

Urea (ref 94, 101, 107)

Urea has been described as bitter and sour. It is used in veterinary medicine as a nutritional source, as a diuretic and as an antiseptic. Ammonia, a urea derivative, is fatally toxic at a concentration of 0.8 g/liter, which implies that solutions of 2.35-M urea may be harmful if ingested in large quantities. Solutions of 0.82- to 1.0-M urea have been used in human gustation experiments. Weaker solutions (eg, 0.05 M) should be safe for marine mammal studies.

Mannose (ref 96, 99, 102-104, 121)

Different isomers of mannose have different tastes. β-D-mannose is bitter, α-D-mannose is sweet and L-mannose is slightly sweet to salty. The specific mannose isomers in the samples were not identified in the analysis. Aqueous mannose solutions have components which produce a complex bitter-sweet stimulus. A solution of ten percent mannose would be appropriate for chemoreception work with marine mammals.

TASTE AND TOXICITY OF GLYCINE AND ALCOHOLS

Glycine (ref 95, 102, 108-111)

Glycine is the principal amino acid in sugar cane and is common in foods such as gelatin. Most amino acids have a sweet taste. The toxicity of glycine is unknown, but glycine is probably safe as an experimental compound.

Glycerol (ref 102, 109, 112, 118, 135)

Glycerol is used commercially as a food sweetener. Data from tests on rats and guinea pigs indicate that the single-dose oral toxicity of glycerol appears to be about half that of ethyl alcohol.

Inositol (ref 96, 105, 113, 114)

Inositol (=1, 2, 3, 4, 5, 6-cyclohexanehexol), which has a sweet taste, is a B-complex vitamin found in wheat germ, lecithin, whole grains, milk, molasses, citrus fruit and brewers yeast. For medicinal uses, daily oral doses of 0.5 to 1.0 grams can be maintained for humans. Test solutions below 0.02 M should be safe.

Arabitol (ref 96)

Arabitol (=1, 2, 3, 4, 5-pantanepentol) has a sweet taste, but toxicity information was unavailable.

Erythritol (ref 96)

Erythritol (=1, 2, 3, 4-butanetetrol) is about twice as sweet as sucrose; no oral toxicity is known. Intravenously-administered erythritol was toxic in dogs in the amount of 5 g per kilogram of body weight.

Mannitol (ref 96, 135)

Mannitol (=mannite or manna sugar), which has a sweetish taste, is used as a pharmaceutical excipient and as a diuretic and diagnostic aid for the kidney function. Oral toxicity data were not available in the literature; however, mannitol should be safe for marine mammal chemoreception studies.

Sorbitol (ref 95, 96, 115, 116, 118, 135)

Sorbitol (=D-glucitol), which is about 60 percent as sweet as sucrose, is found in many berries such as ripe mountain ash, as well as in cherries, plums, pears, apples, seaweed, algae and in blackstrap molasses. Sorbitol is used as a sweetening agent and tablet excipient in drugs. It also has various uses in veterinary medicine. Solutions of up to 2.8-M sorbitol were used in human taste perception experiments, and similar concentrations should be appropriate for marine mammals.

Xylitol (ref 115)

Xylitol (=1, 2, 3, 4, 5-pentanepentol), has a sweet, syrupy taste and possible sour aftertaste. Taste experiments on human subjects used 1.0-M xylitol. Similar concentrations should not harm marine mammals.

TASTE OF SWEET SUGARS

General Comments (ref 119, 121, 136)

The particular isomers of the sugars listed in table 14 were not determined in the analysis, but the isomers with the sweetest tastes are listed. The sugars in table 14 are also polyhydric alcohols, but differ in chemical structure and properties from those in table 13. The slow aqueous interconversion between the α- and β-forms of sugar suggests that sugar solutions should equilibrate for at least three hours before use in chemoreception experiments. Equilibrated solutions will produce uniform stimulus quality. The sugars listed in table 14 are not considered harmful in normal use.

Erythrose (ref 96)

D-erythrose (=(R)-2, 3, 4-trihydroxybutanal) is described as a syrup, and L-erythrose, as a sweet tasting syrup. Toxicity information was unavailable for erythrose.

Galactose (ref 96, 117-120)

Galactose, which has a sweet taste, is found in many foods. A dosage of 40 g of galactose has been used as a liver function test.

In taste tests on humans, 1.5-M solutions of galactose (roughly 27 percent) were used. This concentration also should be safe for gustation experiments on marine mammals.

Glucose (ref 96, 115, 117-119, 121, 136, 137)

Glucose is an important nutrient commonly found in foods and generally is described as sweet. Glucose was observed by some researchers to produce a bitter side ta te. L-glucose is slightly salty. A solution of 2.0-M glucose (roughly 36 percent) was used in taste tests on human subjects and should be safe for marine mammal chemoreception experiments.

Lactose (Ref 96, 117-119, 122)

Lactose is faintly sweet but not as sweet as the other

sugars in table 14. Solutions of 0.6-M lactose (about 21 percent) were used as stimuli for human chemoreception studies and the same concentration is suggested for experiments on marine mammals.

Mannose

Mannose was discussed above in the section on Taste and Toxicity of Sour and/or Bitter Substances.

Xylose (ref 96, 115, 118, 123)

D-Xylose has a very sweet taste tinged by slightly unpleasant components in its taste quality. It is used as a diagnostic aid and as a diabetic food. Xylose concentrations up to 2.5 M have been used in human perceptual studies; similar concentrations should be safe for marine mammals.

TOXICITY AND DISCUSSION OF COMPOUNDS WHICH ARE INSOLUBLE IN WATER AND/OR UNSAFE FOR CHEMORECEPTION EXPERIMENTS

The compounds listed in table 15 are inappropriate for marine mammal chemoreception experiments. p-Hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic and palmitic acids are carcinogenic, mutagenic and tumorigenic, respectively (ref 95, 99, 116, 124, 126, 127). Because tests suggested that aromatic diols generally should be considered potential mutagens and carcinogens (ref 126), 3,5-dihydroxybenzoic acid also may be unsafe. Stearic, oleic, myristic and arachidic acids and cholesterol are insoluble in water (ref 96, 99).

TOXICITY OF NITROGEN HETEROCYCLES

The basic fractions contained several nitrogenous compounds, including tryptamine, an indole derivative. Indole and skatole (3-methylindole), which have strong odors, are common metabolic products (ref 138-144). They become toxic to mammals at species-dependent dosages (ref 72-91).

Sokolov et al used a 0.01-percent solution of indole in seawater for chemoreception experiments with dolphins, but does not mention toxic reactions (ref 7). Cattle have been given 0.05 grams of indole per kilogram of body weight without ill effects (ref 74). Indole should be handled with great care. Indole concentrations for marine mammal experiments should not exceed 0.1 percent (ref 91).

Skatole was more toxic than indole when administered orally to cattle (ref 74), but an exact mammalian toxicity threshold was unreported.

RECOMMENDED EXPOSURE

Table 16 summarizes toxicity and exposure information on compounds identical or similar to those found in the samples. The listed safe levels are maxima. The levels are probably conservative, however, because considerable ingestion of the solution is assumed whereas ingestion volumes of test solutions have not been measured so far in marine mammal chemoreception studies. Also, the actual toxicities of these chemicals to marine mammals are unknown.

Apart from Soviet reports, little information is available on systematic exposure of marine mammals to chemicals.

COMPOUND	GENERAL pH RANGE	ESTIMATED MAXIMUM SAFE LEVEL OF ORAL EXPOSURE	LITERARY REFERENCE
Lactic Acid	Acidic	0.7%	93
Succinic Acid	Acidic	Not found	
Phosphoric Acid	Acidic	0.05%	100
Urea	Basic	0.05M	94, 101 107
Mannose	Neutral	10% (Non-toxic)	103
Glycine	Acidic	2.0M (Non-toxic)	109
Glycerol	Acidic	Non-toxic	112
Inositol	Acidic-Neutral	0.02M	113
Arabitol	Neutral	Not found	
Erythritol	Neutral	Not found	
Mannitol	Neutral	Non-toxic	
Sorbitol	Neutral	2.8M	118
Xylitol	Neutral	1.0M (Non-toxic)	115
Erythrose	Neutral	Non-toxic	
Galactose	Neutral	2.5M (Non-toxic)	118
Glucose	Neutral	2.0M (Non-toxic)	118
Lactose	Neutral	0.6M (Non-toxic)	118
Xylose	Neutral	2.5M (Non-toxic)	118
Indole	Basic	0.01%	74
Skatole	Neutral-Basic	0.005%	74

Table 16. Recommended concentrations of compounds for use in chemoreception experiments.

For future work with the compounds discussed here, the following points are relevant to the NOSC chemoreception program:

1. The values listed in table 16 are estimated maximum concentrations for oral exposure to marine mammals.

2. Marine mammals' exposure to chemicals toxic to other animals should be limited. If greater concentrations are used, the physical condition of the test animals should be monitored closely.

3. Test mannose, erythrose, glycerol, erythritol, arabitol and mannitol for taste reception using solutions of 10 percent.

4. The chemicals listed in tables 12 through 14 are considered suitable for use in chemoreception experiments.

5. Phosphoric acid is probably also suitable for chemoreception experiments, but requires special handling and storage (ref 145). Phosphoric acid should not be used, therefore, if other chemicals are available (ref 145).

6. p-Hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic, and palmitic acids are hazardous to humans and should not be used in marine mammal chemoreception studies.

7. Because related aromatic diols are mutagens (ref 126, 107), use of 3,5-Dihydroxybenzoic acid in marine mammal chemoreception studies is not recommended.

8. Stearic, oleic, myristic and arachidic acids and cholesterol are unsuitable for marine mammal chemoreception studies because they are insoluble in water.

9. Monitor animal health carefully throughout experiments. Avoid prolonged exposure to known toxic compounds, even in very dilute solutions.

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APPENDIX A: DISCUSSION OF SAMPLE COLLECTION TECHNIQUES

Chemical analyses of water deposited into a plastic bag, plastic cup and vial were performed using the gas chromatograph and mass spectrometer apparatus described in reference 13. All containers were new and unused. The chromatograms showed five large peaks in the plastic bag wash, all identified as grease. The chromatograms of the water that rinsed the plastic cup and vial also showed many peaks. Impurities in the containers for some of the samples added to the level of background peaks so that there was a major interference with the sample analysis.

Several compounds were identified in the water rinse of a rubber catheter, the results of which are shown in table A-1. This level of contamination suggests that urine samples intended for chemical analysis should not be obtained with a catheter made of this material. A teflon catheter, if available and properly cleaned, may be acceptable.

In the future, it is recommended that all glass containers with teflon or foil lid liners be used. All glassware should be cleaned by hand, and after washing with HPLC-Grade solvent (acetone, hexane), the container should be given a final wash in dilute HF (1-3%). It is advantageous that a reasonable quantity of the cleaned containers be obtained from the laboratory for sample collection. This approach would save the effort needed in confirming that all solvents including the water were organic free.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		OV-101
Palmitic Acid	0.30	1.0	Palmitic Acid	1.0
Stearic Acid	1.0	0.0	Stearic Acid	0.57
Myristic Acid	0.063	0.0		
Inositol	0.038	0.0		

Table A-1. Principal chemical components in the acidic and neutral fractions of a water extract of a rubber catheter. The estimated relative abundances are indicated with those of the most abundant component for each chromatographic column type, equal to one.

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